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Strapline: Cancer

# *Mutations differ in normal and cancer cells*

***[Draft standfirst]***

What determines whether genetic mutations initiate cancer formation? Analyses of healthy cells in the human oesophagus reveal that a high level of genetic alterations arise as people age yet this doesn't usually lead to cancer.

***[Standfirst of ~190–225 characters, including spaces.]***

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Errors in DNA replication can alter a cell's DNA sequence. If such alterations occur early in embryonic development, the changes are inherited by all of an organism's cells. But if these alterations arise later in adult life, it is more difficult to track any such change in a small number of cells in a specific tissue, so the extent of alteration in normal tissues is poorly understood. It is thought that cancer initiates when cells acquire a minimum compendium of genetic alterations needed to initiate tumour formation. Understanding when such initiating mutations occur in normal cells is crucial to enable the reconstruction of the early events that lead to cancer. Writing in *Nature*, Yokoyama *et al*.[1], and writing in *Science*, Martincorena *et al*.[2] report their respective analyses of the extent of mutations present in human epithelial tissue from the healthy oesophagus and their assessments of how this illuminates our understanding of the processes that drive cancer development.

Martincorena and colleagues sequenced 74 cancer-associated genes in 844 tissue samples taken from the upper oesophagus of nine healthy donors who differed in their gender, age and lifestyles. For 21 of these samples, the authors also determined the whole-genome sequences. A previous study[3] assessing mutations in healthy skin cells reported between around two to six mutations per million nucleotides of DNA. By contrast, Martincorena and colleagues report that the mutations in oesophageal cells arose at a level approximately ten-fold lower than the level reported in skin. This difference was unsurprising because skin cells are exposed to more DNA-damaging agents, such as ultraviolet light, than oesophageal cells. Instead, the surprise was that healthy oesophagus had more mutations in cancer-associated genes than were found in healthy skin. Moreover, at least a subset of these altered genes was under strong positive selection, meaning that the alterations in the genes promoted cell proliferation, giving rise to clonal expansion of such cells. The authors found that the donors' samples had an average of approximately 120 different mutations in

*NOTCH1*, a known cancer-associated gene, present per $cm^2$ of normal oesophageal tissue. Several of the mutations were of the same type that occurs in the cancer of the upper oesophagus called oesophageal squamous cell carcinoma (OSCC).

Yet despite these similarities, there are striking differences between the cell clonal expansion of the normal oesophageal epithelium and OSCC. Normal and cancer clones seem to be driven by mutations in different genes. *NOTCH1* was the most-frequently mutated gene in healthy oesophageal cells, whereas a previous study[4] reported that *NOTCH1* was mutated only in around 10% of OSCC. Mutations in the cancer-promoting gene *TP53* are found in more than 90% of OSCC cases[4] but they were present at a much-lower frequency in normal oesophageal samples.

In normal cell clones, the prevalent mutational signature — the type of nucleotide changes and the DNA context where these occur, was typical of physiological processes such as those associated with gene transcription and ageing. Consistent with this, the overall number of mutations, as well as the number of mutations in cancer-associated genes and the size of the clones were greater in the samples from older people than in the samples from younger people. By contrast OSCC is mostly dominated by mutational signatures associated with other mutation-causing agents such as, for example, cigarette smoking, alcohol intake or an enzyme called APOBEC that can modify DNA[5]. Another OSCC hallmark is chromosomal instability, which causes frequent gene loss or gain[4]. By contrast, Martincorena and colleagues observed low levels of chromosomal instability in healthy oesophageal cells.

Yokoyama and colleagues found similar results to those of Martincorena and colleagues. Yokoyama *et al*. analysed 682 samples of healthy and cancerous oesophageal tissue of 139 individuals, who differed in age and risk of developing OSCC. The authors used a combination of approaches to determine DNA sequences ranging from whole-genome sequencing and whole-exome (protein-coding regions) sequencing to re-sequencing of specific genes. Yokoyama and colleagues found mutations in normal samples from cancer***[please check as normal seems to contradict the use of 'cancer' Mary, a cancer patients will have plenty of normal tissue around the cancer and they wanted to see if this normal tissue had the same cancer mutations]*** and healthy individuals. The number of mutations in the normal oesophagus increased with an increase in age, clone size and exposure to known cancer-risk factors.

Yokoyama *et al*. also observed most of the same differences between normal and cancer cell clones that were noted by Martincorena and colleagues. Using two different computational approaches, they identified 24 genes that were frequently mutated in the healthy and cancer samples, but only six of these genes were shared between the two groups. As in the study of Martincorena and colleagues, Yokoyama *et al*. also found that *TP53* and *NOTCH1* were the most commonly mutated genes in cancer and healthy samples, respectively. The age-related mutational signature was prevalent in normal oesophageal cells, especially those from individuals with a lower risk of developing OSCC. By contrast, mutational signatures associated with APOBEC activity or alcohol intake were prevalent in cancer samples and detectable in normal samples from higher cancer-risk individuals. They

detected few chromosomal alterations in normal samples and confirmed the high level of chromosomal instability in OSCC.

Both studies offer insights into the evolution of healthy tissues as people age and prompt speculations on how this might relate to the development of cancer. The clonal expansion of normal oesophageal cells after cancer-promoting genes are mutated seems to be necessary but not sufficient to drive cancer, and something else needs to happen to the cells for tumours to form. For example, gaining a large-enough number of alterations in cancer-promoting genes might be needed. In both studies, normal clones carried few mutations present in all the cells and many of the cancer-promoting mutations were often found in spatially distinct subclones. This suggests that none of the normal cells had acquired enough cancer-promoting alterations to start cancer formation. Other missing factors needed to drive cancer formation might be of environmental origin.

OSCC occurs frequently in Asia and South America but is rare in the Western world[6]. The reasons underlying this geographical distribution are still mainly unknown, but it is thought that lifestyle and environmental factors might have important roles[7,8]. All the donors providing samples for Marincorena and colleagues' study were from the UK, and it is likely that, at least in these cases, the mutated cells lacked the combination of the external factors needed to initiate OSCC. By contrast, the samples analysed by Yokoyama and colleagues were from Japanese individuals and some of their donors were at high risk of developing OSCC. Even in these individuals, however, several normal clones that appeared to have been present for a long time had not developed into cancer. For example, a clonal expansion in a 70-year-old individual with a high risk of developing OSCC probably started with a mutation in *TP53* at that the authors estimated occurred when the individual was 13 years old. For many decades, this cell clone expanded to reach an area of 7 $mm^2$ but it did not develop into cancer, confirming that additional unknown factors would be required for that to happen.

Another tempting speculation to make concerns the cancer-driving role of *NOTCH1* and other cancer genes that are more frequently mutated in normal tissue than in cancer. Clearly alterations in these genes are not early events in cancer progression. Their relatively high mutation frequency in OSCC might be a consequence of the fact that they are often mutated in normal tissue rather than an evidence of their function as a cancer driver**.** If this is the case, it challenges the idea that common gene alterations in cancer samples indicate genetic changes that are likely to have a cancer-promoting role. Yet this is still the most-common approach used to identify cancer-associated genes[9].

Finally, the role that mutations in genes not involved in cancer might have in the clonal expansion of normal tissues remains to be investigated. Given the partially independent mechanisms that seem to drive the expansion of normal and cancer clones, perhaps mutations in genes involved in other processes, for example genes involved in ageing, might be functionally important in normal clones. Martincorena and colleagues only sequenced well-known cancer-promoting genes, and therefore this type of analysis was not feasible in their study. Yokoyama and colleagues sequenced all protein-coding regions of the genome but only used two well-established computational approaches to find cancer-promoting mutations in normal samples. Unsurprisingly, they found mostly known cancer genes.

Interestingly, they found that the gene *PAX9*, which encodes a transcription factor is significantly***[is this being used in the sense of statistical significance? Yes, but 'at the level of statistical significance' is not used. If you do not like significantly replace with commonly]*** mutated in normal oesophageal tissue, but has not been associated with cancer formation so far. This suggests that a less 'cancer-centric' analysis of genes might reveal other genes that can drive the expansion of clones in normal tissue. We are only starting to map the extent of genetic alterations in normal tissues. The next challenges will be to fully understand their role in healthy tissues and in disease states.

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